SYMPOSIUM REPORT

The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis

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The synaptotropic hypothesis, which states that synaptic inputs control the elaboration of dendritic (and axonal) arbors was articulated by Vaughn in 1989. Today the role of synaptic inputs in controlling neuronal structural development remains an area of intense research activity. Several recent studies have applied modern molecular genetic, imaging and electrophysiological methods to this question and now provide strong evidence that maturation of excitatory synaptic inputs is required for the development of neuronal structure in the intact brain. Here we critically review data concerning the hypothesis with the expectation that understanding the circumstances when the data do and do not support the hypothesis will be most valuable. The synaptotrophic hypothesis contributes at both conceptual and mechanistic levels to our understanding of how relatively minor changes in levels or function of synaptic proteins may have profound effects on circuit development and plasticity.

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The synaptotrophic hypothesis

The synaptotrophic hypothesis states that synaptogenesis is an orderly series of hierarchical processes that culminates in the formation of stable mature synapses (Vaughn *et al.* 1974, 1988; Vaughn, 1989). There is little controversy over this aspect of the hypothesis and it is supported by a mountain of evidence. The more controversial part of the model states that growing neuronal processes extend toward regions where they are likely to find synaptic partners. Vaughn further states that the establishment of synaptic contacts stabilizes growing neuronal processes. Integral to this concept is the idea that growing neuronal processes are dynamic and exploratory. Despite the fact that Vaughn formulated the synaptotrophic hypothesis based largely on electron microscope studies of developing vertebrate central nervous system and spinal cord, he, like Cajal, imagined the fixed structures as participating in a lively dance of exploration during the process of circuit formation.

In particular, Vaughn stated that 'the formation of synaptic junctions may take place as an ordered progression of epigenetically modulated events wherein each level of cellular affinity becomes subordinate to the one that follows. The ultimate determination of whether a synapse is maintained, modified or dissolved would be made by the changing molecular fabric of its junctional membranes. . . . a hypothetical model of synaptogenesis is proposed, and an hierarchical order of events is associated with a speculative synaptogenic sequence. Key elements of this hypothesis are 1) epigenetic factors that facilitate generally appropriate interactions between neurites; 2) independent expression of surface specializations that contain sufficient information for establishing threshold recognition between interacting neurites; 3) exchange of molecular information that biases the course of subsequent junctional differentiation and ultimately results in 4) the stabilization of synaptic junctions into functional connectivity patterns.' (Vaughn, 1989).

A key feature of Vaughn's statement of the synaptotrophic hypothesis is that it incorporates what are classically considered activity-independent and activity-dependent mechanisms. Vaughn postulated that gradients of cell surface or secreted molecules target axons and dendrites to approximately the correct brain

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region, as has been demonstrated with molecules such as ephrins (O'Leary & Wilkinson, 1999; Feldheim *et al.* 2000). Consistent with Sperry's chemoaffinity hypothesis, he also suggested that cells have mechanisms to detect specific concentrations of extracellular cues, as has also been demonstrated. Once axons and dendrites have grown into approximately the correct region, there is an 'exchange of molecular information that biases the course of subsequent junctional differentiation'. In other words, synaptic transmission triggers events which either increase or decrease the likelihood that synapses would be maintained. Vaughn anticipated that anterograde and retrograde signals cooperate to stabilize synaptic contacts. Considerable effort has been directed to identify 'synaptotrophins' that might mediate the anterograde and retrograde signalling events (Snider & Lichtman, 1996). We now classify mechanisms of trans-synaptic information exchange as those mediated by direct molecular interactions and those mediated by diffusible signals. A growing list of cell adhesion molecules provides candidates for direct molecular links for both anterograde and retrograde signalling. Adhesion molecules may mediate the initial target recognition for synapse specification, and promote pre- and post-synaptic differentiation and long-term stabilization as synapse maturation proceeds. Diffusible signals, involving traditional anterograde synaptic transmission and poorly understood retrograde factors, however, allow pre- and postsynaptic neuronal activity to directly influence molecular mechanisms of synapse maintenance. Synaptic transmission could include co-released peptides or the contents of dense core vesicles, which are prevalent in nascent synapses (Ziv & Garner, 2004). The identification of retrograde signals has been more elusive, and may range from released molecules, such as brain-derived trophic factor (BDNF) or protein complexes which stretch across the synaptic cleft, such as ephrins and Ephs, neuroligins and neurexins or cadherins.

Evidence supporting the synaptotropic hypothesis is strongest in sensory projections including sensory cortex, but neurons that receive an abundance of converging inputs from diverse modalities or with diverse patterns of input may not show dendritic growth plasticity that reflects synaptic input activity.

It is interesting to note that Vaughn specified that 'epigenetic influences' play a prominent role in synapse formation. At the time 'epigenetic' referred to influences that were not directly affected by gene expression and are now referred to as 'activity-dependent'. With the identification of hundreds of genes which are induced by neuronal activity (Nedivi, 1999), as well as the activity-dependent trafficking of cell surface adhesion molecules (Cantallops & Cline, 2008), it is now clear that the attempted distinction between 'activity-dependent' and 'activity-independent' or between 'epigenetic' and genetically determined events in neuronal development is a false dichotomy.

Although the synaptotrophic hypothesis is often simplified as 'Synaptic input governs the elaboration of the dendritic arbor', a broader statement of the hypothesis derives from the idea that an exchange of information between pre- and postsynaptic elements biases the course of subsequent synapse formation and stabilization. Synaptic activity can result in long-term changes in gene expression patterns (Ghosh *et al.* 1994), so even calcium-dependent changes in transcription which subsequently have a bearing on synaptic signalling can reflect synaptotrophic mechanisms. For instance, calcium influx downstream of glutamatergic synaptic activity leads to the activation of transcription factors including CREB, Crest and MEF2 (Flavell *et al.* 2006). MEF2 was recently shown to increase transcription of genes which in turn regulate excitatory synapse numbers (Flavell *et al.* 2006). Furthermore, acute application of a classical guidance molecule, semaphorin, to hippocampal slices affects synaptic transmission (Sahay *et al.* 2005), so experiments demonstrating a role for guidance molecules in dendritic arbor development could provide support for the synaptotrophic hypothesis as well (Polleux *et al.* 2000). The point is that, in many cases, experiments haven't been done to test whether some of these classical guidance/adhesion molecules might affect neuronal development through an effect on synapse formation and stabilization.

The strongest evidence against the synaptotropic hypothesis comes from studies of the munc 18 knock-out mice (Verhage *et al.* 2000). Although these animals die without taking a breath, it is amazing that they develop a brain with grossly normal structure including synapses without calcium-dependent synaptic transmission. It is possible that non-vesicular release mechanisms operate and are up-regulated in the absence of calcium-dependent transmission. Reports of molecules that affect dendritic arbor structure without affecting synaptic input (Moore *et al.* 2002) and reports that synapse density can be modified, for instance by BDNF, without affecting dendritic arbor structure (Sanchez *et al.* 2006) also suggest that dendritic arbor development can develop independently of coordinated synapse development. It will be important to identify these mechanisms.

Dendrite arbor development and synapse formation/maturation are concurrent

A key observation from Vaughn's original studies is that dendrite arbor development and synapse formation/maturation are concurrent (Fig. 1). This provides the foundation of the synaptotrophic hypothesis, but has also made it a challenge to test the hypothesis

experimentally. Since the synaptotrophic hypothesis states that synaptic inputs regulate dendritic arbor development, evaluation of the hypothesis requires an understanding of the processes of synaptogenesis and synapse maturation as well as an accurate description of dendritic arbor development. Now that we have sufficiently detailed information describing these complex events from a number of systems including the retinotectal system of both *Xenopus* and Zebrafish, it has been possible to test whether synaptic inputs control morphological development (Niell *et al.* 2004; Haas *et al.* 2006). Below we will review the events underlying arbor development and synapse maturation, before discussing tests of the synaptotrophic hypothesis.

Process of dendritic arbor development

In vivo time-lapse imaging has demonstrated that dendrites of CNS neurons grow by the highly dynamic addition and retraction of fine branches (Wu & Cline, 1998; Wu *et al.* 1999; Wong *et al.* 2000; Sin *et al.* 2002; Wong & Ghosh, 2002; Niell *et al.* 2004). Imaging Green Fluorescent Protein (GFP)-labelled optic tectal cells at 3 min intervals indicates that the average lifetime of tectal cell dendritic branches is about 10 min. A minority of branches is maintained for longer periods and they have lifetimes ranging from 10 min to hours to days. The accuracy with which the lifetimes of dynamic structures can be estimated depends on the frequency of image collection and of course requires that the imaging interval be sufficiently frequent that the dynamic structure can be observed at least twice. While most newly added branches rapidly retract, a small fraction is maintained and extends to become long-lasting components of the arbor (Wu & Cline, 1998). The extensive turnover of dendritic filopodia and their wide coverage of local 3D space over time suggest that these newly added branches may actively sample the local environment for appropriate presynaptic contact sites. Successful search outcomes for detecting appropriate synaptic partners, and subsequent establishment and maintenance of 'functionally correct' synapses, may then confer a longer lifetime on these branches (Ziv & Smith, 1996; Wong *et al.* 2000; Cline, 2001; Wong & Ghosh, 2002; Portera-Cailliau *et al.* 2003; Hua & Smith, 2004; Konur & Yuste, 2004; Niell *et al.* 2004; Hua *et al.* 2005). Mature synapses may stabilize local morphology by creating nucleation sites for cytoskeleton binding proteins.

Steps of synaptogenesis and synapse maturation

1. Formation of initial contacts by cell–cell adhesion. An initial adhesive event, possibly mediated by integrins, cadherins, or wnt/frizzled signalling (Yamagata *et al.* 2003) can occur between dynamic axonal filopodia and dynamic dendritic filopodial extensions or on dendritic branches, for instance in the case of *en passant* synapses. Dynamic axonal and dendritic filopodia may increase opportunities and sampling territory for forming initial contacts. Very rapid *in vivo* imaging of optic tectal cells, in which images were collected every 10 or 30 s suggests that dendritic filopodia may be added and retracted with lifetimes in the order of minutes. The transient nature of these highly dynamic dendritic filopodia indicates that whatever cellular mechanisms stabilize the filopodia are rarely triggered, suggesting that the initial adhesive events are rare.

2. Conversion of adhesive contact to a nascent synapse. Nascent synapses are characterized electrophysiologically by the presence of postsynaptic NMDA-type glutamate receptors but not AMPA receptors. NMDARs are prevalent in extrasynaptic plasma membrane (Thomas *et al.* 2006) and their lateral movement within the membrane or insertion into the plasma membrane from intracellular stores may be triggered by an adhesive contact. For instance, EphrinB-EphB receptor signalling may recruit NMDARs to synapses (Dalva *et al.* 2000). Alternately, there may be a sufficient density of NMDARs in the dendritic plasma membrane, so that their activation by glutamate is sufficient to trigger the next step in synaptogenesis: recruitment of AMPARs and other components of the postsynaptic density. Presynaptically, nascent synapses have a sparse assembly of synaptic vesicles, relatively few docked vesicles and a poorly defined presynaptic active zone. The prevalence of

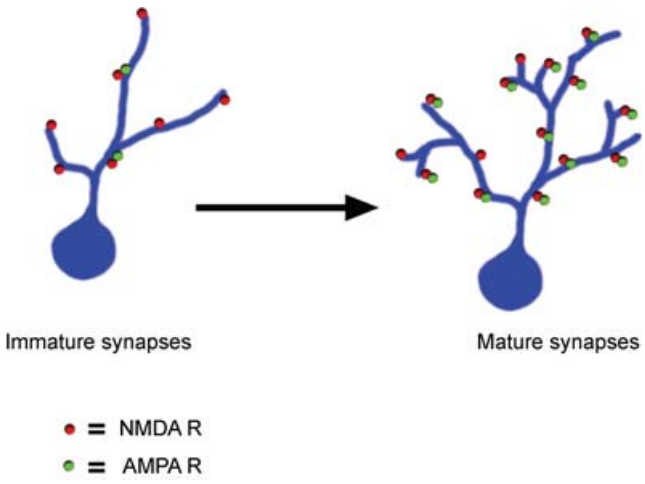

Figure 1. Dendritic arbor growth and synapse maturation are concurrent
The diagram shows an immature neuron with a simple dendritic arbor and excitatory synapses which are predominated by NMDA-type glutamate receptors. As the neuron matures, the dendritic arbor becomes more complex and the synapses mature by adding AMPA-type glutamate receptors.

dense core vesicles is relatively high in nascent synapses, and their fusion with the active zone is thought to deliver structural proteins to the presynaptic site (Ziv & Garner, 2004).

3. Synapse maturation. Maturation of glutamatergic synapses is characterized electrophysiologically by an increase in the amplitude of AMPA receptor-mediated synaptic transmission and a change in the NMDAR subunit composition which has a bearing on the time-course of the synaptic response, synaptic intergration and the calcium conductance of the receptor (Carmignoto & Vicini, 1992; Hestrin, 1992; Bellone & Nicoll, 2007). Studies in many experimental systems, including the tadpole optic tectum, indicate that newly formed synapses are mediated by NMDA type of glutamate receptors and that AMPA receptors are added to synapses as they mature (Liao *et al.* 1995; Wu *et al.* 1996; Durand *et al.* 1996; Isaac, 1997; Cantallops *et al.* 2000; Zhu *et al.* 2000). Synapses with only NMDAR are 'silent' at resting potential due to the voltage-dependent block of the NMDA receptor channel, and the addition of AMPAR to synapses renders them functional at resting potentials. Similarly, the fraction of silent synapses, in which transmission is mediated solely by NMDARs, is high in early stages of synapse formation and decreases as synapses and neurons mature, due to the insertion of AMPARs at synaptic sites. Consequently, the biophysical properties of NMDAR-mediated synaptic responses, the fraction of silent synapses and the ratio of AMPA to NMDA receptor-mediated transmission can be used as indicators of synaptic maturity.

In many systems, the AMPARs that are initially added to silent synapses are calcium permeable, because they lack the GluR2 subunit, and the calcium-permeable AMPARs are later replaced by receptors including the GluR2 subunit, which are calcium impermeable (Zhu *et al.* 2000; Aizenman *et al.* 2002; Kumar *et al.* 2002; Eybalin *et al.* 2004; Ho *et al.* 2007; Migues *et al.* 2007). The transient presence of GluR2-lacking AMPARs may have several effects of the course of synapse maturation and developmental synaptic plasticity (Aizenman *et al.* 2002; Ho *et al.* 2007). Although there are important differences in the magnitude, time-course and synaptic trigger events that result in calcium entry through GluR2-lacking AMPARs and NMDARs, calcium entry through GluR2-lacking AMPARs may promote subsequent steps of synapse maturation, potentially comparable to the role NMDAR-mediated calcium entry is thought to play.

In addition to the electrophysiological parameters mentioned above, synapse maturation includes the assembly of the postsynaptic density, a complex and dynamic array of proteins with both structural and signalling functions (Sheng & Hoogenraad, 2007). Although the recruitment of AMPA receptors is typically the signature for synapse maturation, because it represents an increase in synaptic strength, the quintessential function of the synapse, neurotransmitter receptor recruitment is just one of many critical events in the assembly of the complex postsynaptic density. Other events include coalescence of proteins into complexes that link to the cytoskeleton. It is interesting to note that very few postsynaptic density (PSD) proteins are dispensable and increases or decreases in copy number of genes or postsynaptic protein levels often show phenotypes. For instance, PSD95 levels seem to be tightly regulated by a variety of mechanisms, and manipulations that affect postsynaptic PSD95 show structural and functional phenotypes. Although many investigations of PSD95 have been spurred by the availability of reliable reagents, PSD95 may not play a unique role in regulating postsynaptic function. It seems that the stoichiometry of most PSD proteins is tightly regulated and that disruptions in the stoichiometry of several PSD proteins affect synaptic function and signalling (Sheng & Hoogenraad, 2007).

Synapse maturation includes changes in the presynaptic element including recruitment of presynaptic proteins into the terminal specialization (Ziv & Garner, 2004), recruitment of synaptic vesicles and often an increase in the reliability of synaptic transmission. It is noteworthy that the establishment of pre- and postsynaptic specializations is highly regulated spatially and temporally. Pre- and postsynaptic elements are exactly apposed to one another. Similarly, they develop in a temporally coordinated fashion. Clearly, some type of trans-synaptic communication regulates pre- and postsynaptic development. Recent studies suggest that top candidates for this type of trans-synaptic anterograde and retrograde communication include neurexin/neuroligins/PSD95 (Futai *et al.* 2007), and cadherin/catinen (Bamji, 2005).

Tests of the synaptotrophic hypothesis

Evidence that interference with each or any of these steps in synaptogenesis and synapse maturation inhibits dendritic arbor development would provide support for the synaptotrophic hypothesis.

Adhesion molecules

Evidence for a role of adhesive molecules in synapse formation is convincing (Yamagata *et al.* 2003). For instance, some adhesive molecules, such as neuroligins and their binding partners the neurexins, the B ephrins and their binding partners EphRs may also serve to promote differentiation of pre- and postsynaptic elements (Scheiffele *et al.* 2000; Yamagata *et al.* 2003; Chubykin *et al.* 2007). These adhesion molecules may play a role in synapse formation and maturation by virtue of the fact that they recruit pre- and postsynaptic density proteins

to the synaptic specialization and are thereby critical for the assembly of the synaptic specialization. Recent evidence suggests that an interaction between neuroligin and PSD95 is important for synapse maturation through the recruitment of glutamate receptors (Futai *et al.* 2007). The synaptotrophic hypothesis would predict that adhesion molecules which play a principle role in constructing the synapse would also play a critical role in regulating the development of the dendritic arbor, for instance, as has been demonstrated with cadherins (Ye & Jan, 2005).

Glutamatergic synaptic transmission governs structural plasticity

Blocking either AMPA- or NMDA-type glutamate receptors decreases optic tectal cell dendritic arbor growth in *Xenopus* tadpoles *in vivo* (Rajan & Cline, 1998; Rajan *et al.* 1999; Sin *et al.* 2002) and in isolated retina (Wong *et al.* 2000), and blocks visual stimulation-induced dendritic arbor growth in *Xenopus* optic tectal cells (Sin *et al.* 2002). These data suggest that glutamatergic synaptic transmission controls dendritic arbor growth by regulating branch dynamics (Sin *et al.* 2002). We propose that trafficking AMPARs into synapses stabilizes newly added branches, which are sites of nascent synapses, and provides a substrate for further branch addition, so that dendritic arbors gradually grow and become more complex through an iterative process of branch addition and stabilization (Hua *et al.* 2005); however, the widespread reduction of glutamatergic transmission reduces many activity-dependent processes, including release of neurotrophic growth factors and differential patterns of activity required for competition-based developmental plasticity. Consequently, we used a different strategy to restrict interference of synapse maturation to individual neurons and tested effects on dendritic arbor growth within a brain experiencing normal circuit activity. The logic was to interfere with trafficking of AMPARs into nascent synapses to limit the progression from immature synapses to mature synapses with a strong AMPAR component. Previous work has demonstrated that AMPARs are trafficked into synapses from intracellular sites and from extrasynaptic membrane. The cytoplasmic tails of different AMPAR subunits govern the regulation of AMPAR trafficking into synapses (Shi *et al.* 2001). Expression of peptides corresponding to the cytoplasmic tails of AMPAR subunits interferes with experience-dependent plasticity in barrel cortex and amygdala (Takahashi *et al.* 2003; Rumpel *et al.* 2005), suggesting that trafficking of AMPARs into synapses is required for synaptic plasticity. However, since trafficking of AMPARs into synapses may escort other components of the PSD into developing synapses, it may not be AMPARs per se that are essential for the plasticity, but rather the assembly of the PSD which is required for synapse maturation and plasticity. Expression of non-conducting, mutant AMPARs (Shi *et al.* 2001) controlled for this possibility since the mutant 'pore-dead' AMPARs interfered with synaptic transmission and synaptic plasticity, based on a point mutation in the GluR2 channel, but probably associates with the normal protein partners as the wild type receptor.

Expressing peptides corresponding to the cytoplasmic tails of AMPARs in *Xenopus* optic tectal neurons decreased the amplitude but not the frequency of spontaneous excitatory synaptic currents (Fig. 2), suggesting that the peptides blocked the increase in AMPARs at synapses that occurs with synaptic maturation (Haas *et al.* 2006). Based on this, we expressed the peptides in individual optic tectal neurons and collected images of the neurons once a day over 4 or 5 days. The peptide-expressing neurons had long, sparsely branched dendritic arbors, with relatively few side-branches extending from the primary dendrites. How could such a morphological phenotype arise from decreased AMPAR trafficking into nascent synapses? One hypothesis is that the stabilization of newly added branches from primary dendrites is prevented by blocking synapse maturation. This would predict that branch lifetimes would be shorter in neurons expressing the peptides compared to control neurons. To test this, we collected images every 2 h over a 6 h period. Analysis of the two images allowed us to identify and monitor the dynamic behaviour of every branch in the dendritic arbor. This analysis demonstrated that peptide-expressing neurons had relatively more branches with the shortest detectable lifetime. A relatively brief 4 h period of enhanced visual stimulation increases the dendritic arbor growth rate of optic tectal neurons (Sin *et al.* 2002). Importantly, we found that the experience-dependent structural plasticity normally seen in optic tectal neurons following visual stimulation is completely blocked by expression of the C terminal peptides of AMPARs (Fig. 3). This series of experiments demonstrated that dendritic arbor development under normal conditions and in response to enhanced visual experience requires trafficking of AMPARs into synapses. This is clear support for the synaptotrophic hypothesis.

It is possible that AMPAR trafficking into synapses may escort other proteins into the PSD, so that interfering with trafficking of AMPARs may hinder assembly of the postsynaptic density, as opposed to a direct role of AMPAR-mediated synaptic transmission. Therefore, a more conservative interpretation of the experiments is that postsynaptic development is required for dendritic arbor development and experience-dependent plasticity. This is still consistent with the synaptotrophic hypothesis, since the deficiency is in synapse maturation, whether it comes about by interefering with AMPAR trafficking

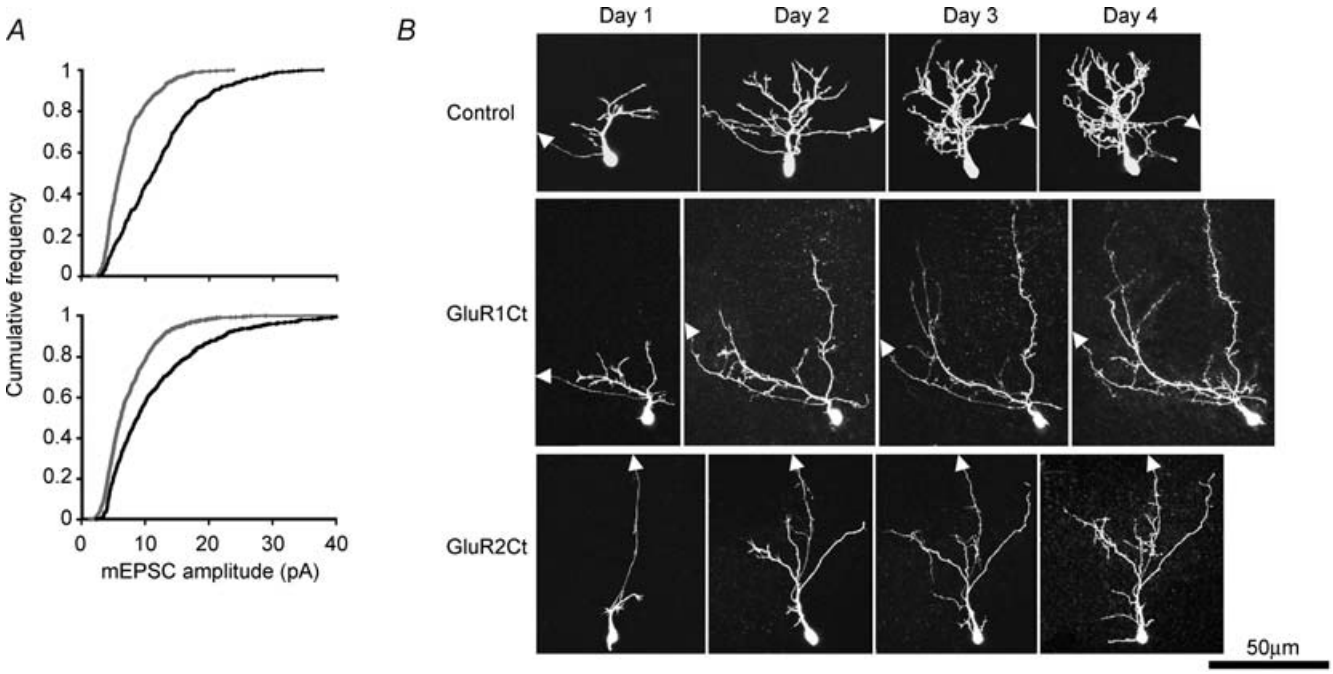

Figure 2. Expression of peptides corresponding to the C terminal of glutamate receptor subunits decreases the amplitude of spontaneous miniature synaptic currents (*A*) and alters the dendritic arbor development of optic tectal neurons (*B*)
Gray lines in *A* are data from neurons expressing GluR1 (top) and GluR2 C terminal peptides (bottom). Black lines are from GFP-expressing control neurons. Adapted from Haas *et al.* (2006).

or assembly of the postsynaptic density. A future challenge is to address whether receptor trafficking, as opposed to postsynaptic scaffold assembly, is required for dendritic arbor development, since assembly of the post-synaptic protein matrix is intimately associated with synaptic transmission and visa versa. An example of the dilemma of sorting out the requirement for receptor trafficking per se from synapse assembly comes from studies of the TARPs (transmembrane AMPA receptor regulatory proteins) (Chen *et al.* 2000; Tomita *et al.* 2005; Osten & Stern-Bach, 2006). The TARPs, including the founding member of the family, Stargazin, are an obligate accessory subunit of AMPARs. In the absence of TARPS, AMPARs are not trafficked into synapses (Chen *et al.* 2000; Tomita *et al.* 2005; Osten & Stern-Bach, 2006). Stargazin binds PSD95 (Bats *et al.* 2007), which in turn binds NMDAR. Given the binding of NMDARs and PSD95 to other postsynaptic density proteins (Sheng & Hoogenraad, 2007), it is straightforward to anticipate that trafficking of these AMPAR accessory proteins may be important

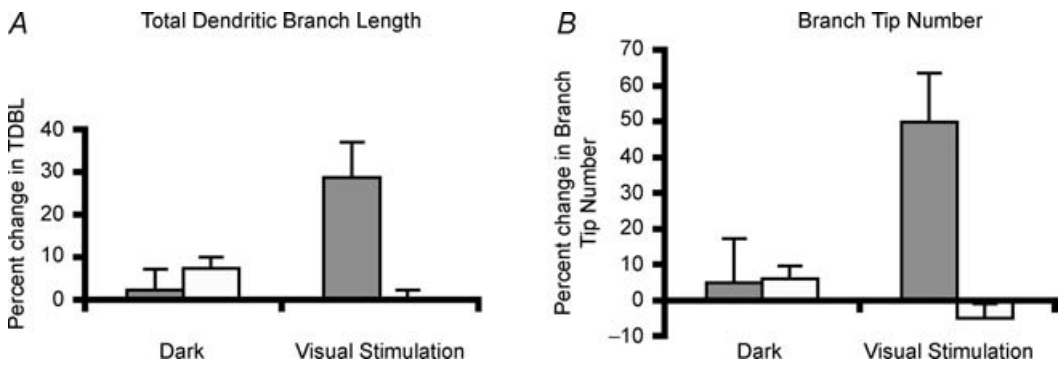

Figure 3. Blocking glutamatergic synapse maturation prevents experience-dependent dendritic arbor growth
Control GFP-expressing neurons increase the relative rate of dendritic arbor growth as a result of 4 h of enhanced visual experience. Neurons expressing the AMPAR C terminal peptides do not respond to the visual stimulation with an increased growth rate.

for assembly of the postsynaptic density. One interesting prediction is that mutant forms of TARPs that disrupt AMPAR trafficking to the synapse would affect structural plasticity and experience-dependent synaptic plasticity. Recent studies indicate that TARPs are quite diverse with respect to their effect on AMPAR function (Cho *et al.* 2007; Milstein *et al.* 2007), and are spatially and temporally regulated during brain development, suggesting that particular isoforms might normally influence AMPAR trafficking during synapse maturation.

Postsynaptic disruptions of synaptic maturation affect presynaptic maturation

The synaptotrophic hypothesis states that synaptic inputs affect dendritic arbor development; however, it is clear that postsynaptic expression of AMPAR cytoplasmic tail peptides also has a profound retrograde effect on presynaptic features of synapse maturation (Haas *et al.* 2006). We used ultrastructural methods to test whether interfering with AMPAR trafficking into synapses, which decreased the amplitude of spontaneous postsynaptic AMPAR currents, consistent with a cell autonomous effect of maturation of the postsynaptic site, also affected maturation of the presynaptic element. By comparing ultrastructural features of synapses in young and more mature *Xenopus* tadpoles we identified several features of developing synapses. A consistent measure that correlated with synapse maturation was the ratio of the area occupied by clustered presynaptic vesicles relative to the area of the presynaptic bouton. Synapses in brains of younger tadpoles have relatively less presynaptic area occupied by clustered vesicles, whereas more presynaptic bouton area is filled with clustered vesicles in synapses from brains of older animals. Importantly, we find that synapses formed onto neurons expressing AMPAR C terminal peptides have significantly lower synapse maturation measures than synapses on control neurons.

These data suggest that AMPAR trafficking can drive presynaptic maturation. It would be interesting to determine how quickly these changes occur. We expressed AMPA C terminal peptides for 3 days before conducting our experiments; however, time-lapse images of the accumulation of synaptophysin-CFP-labelled synaptic vesicles in retinal ganglion cell axons showed that presynaptic clusters of synaptic vesicles accumulate over the time course of hours (Ruthazer *et al.* 2006).

The EM data also demonstrated that the majority of synapses in C terminal peptide-expressing neurons form on larger-caliber dendrites. In control neurons, most synapses form on small-caliber dendrites, which are located further from soma, but the peptide-expressing neurons have many fewer small-caliber dendrites than controls, because these branches are not stabilized by the formation of mature synapses. Consequently, the terminal dendritic branches in peptide-expressing neurons turn over rapidly and synapses cannot be maintained on these dynamic branches. Despite a paucity of fine branches, synapses still form on peptide-expressing neurons, but they form primarily on the larger-caliber more stable parts of the arbor. This raises the question of whether, during normal arbor development, synapses normally form on the primary dendrites when they are themselves fine terminal branches, and then as the arbor grows and branches thicken, are synapses lost from these sites and re-established on smaller more distal branches? Alternately, synapses may be maintained on primary dendrites, but relatively more synapses form on secondary, and higher order dendrites, so the shift in the distribution of postsynaptic profile areas is the consequence of the addition of synapses principally to branch tips.

In conclusion, the synaptotrophic hypothesis, originally proposed by Vaughn based on insight from EM studies, proposes that synapse formation promotes the further elaboration of neuronal structures and ultimately circuit formation. We review the fundamental mechanisms of synapse formation and maturation and suggest how specific interference with synapse maturation by blocking AMPAR trafficking into developing synapses prevents normal neuronal development (Fig. 4), consistent with the synaptotrophic hypothesis. The synaptotrophic hypothesis has value at the conceptual level and may guide our understanding of how relatively minor changes in levels of synaptic proteins may have profound effects on circuit development and plasticity.

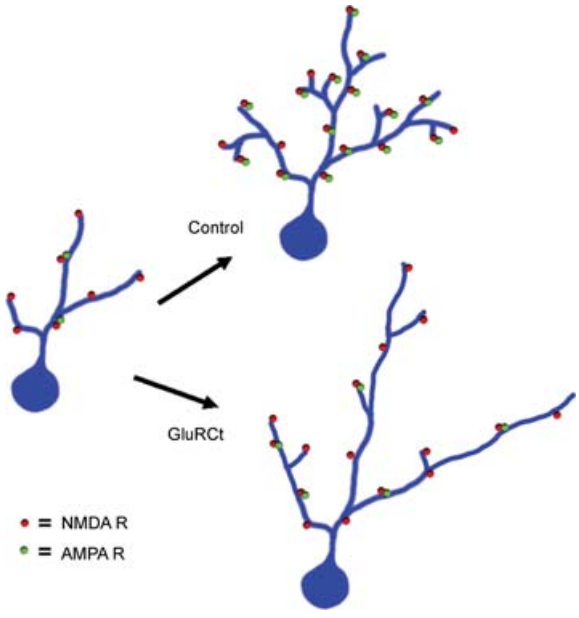

Figure 4. The synaptotrophic hypothesis states that synaptic inputs drive the development of the dendritic arbor
Interfering with synapse maturation prevents normal dendritic arbor growth and would be predicted to affect circuit function.

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